CERTIFICATE OF ANALYSIS

Research use only. Not for use in diagnostic procedures.

LANCE® *Ultra*

Eu-W1024 labeled Anti-phospho-Crosstide (GSK-3α Ser21) Antibody

Product number: TRF0202-D **Lot Number:** 3251202

Product Format: TRF0202-D: 10 µg

TRF0202-M: 100 µg

Manufacturing date: 9/26/2023 **Document version:** 1

Product Information

Antibody: Europium-labeled mouse monoclonal antibody recognizing phospho-Ser21 in peptides derived from glycogen synthase kinase-3 alpha (GSK-3α)

Storage Buffer: 50 mM Tris-HCl (pH 7.4), 0.9% NaCl with 0.05% sodium azide as preservative and 0.1 % BSA

Molecular Weight: 160 000

Stability: This product is stable for at least 24 months from the manufacturing date when stored in its original packaging and at the recommended storage conditions.

Storage Conditions: Store at 4°C.

Quality Control

The QC release specifications are based on spectrophotometric analysis of the labeled antibody. We certify that results meet our quality release criteria.

Labeling Ratio: 5.23
Concentration: 100 µg/mL (0.625 µM)

Recommended Assay Conditions

MSK1: ATP titration

SUGGESTED METHOD:
(Specific applications might require optimization)

Reagent Preparation

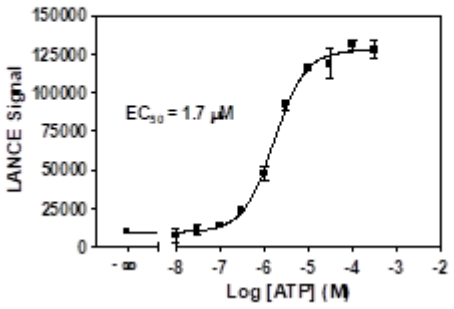
- Prepare 1X Kinase Assay Buffer: 50 mM HEPES pH 7.5, 1 mM EGTA, 10 mM $MgCl_2$, 3 mM $MnCl_2$, 2 mM DTT and 0.01% Tween-20.
- Prepare a 2X MSK1 solution: dilute the enzyme to a concentration of 4 nM in Kinase Assay Buffer. Keep on ice.
- Prepare a 4X mix of U*Light*™-Crosstide: dilute U*Light*™-Crosstide to a concentration of 200 nM in Kinase Assay Buffer.
- Prepare a 4X mix of ATP: dilute ATP to concentrations ranging from 40 nM to 4 mM (serial half-log dilutions) in Kinase Assay Buffer. Keep on ice.
- Prepare 1X Detection Buffer: dilute 0.5 mL of 10X Detection Buffer with 4.5 mL of H_2O.
- Prepare a 4X Stop Solution: prepare a 40 mM EDTA solution in 1X Detection Buffer.
- Prepare a 4X Detection Mix: dilute the Eu-anti-phospho-Crosstide antibody to a concentration of 8 nM in 1X Detection Buffer.

Protocol

- Pipet 5 µL of 2X MSK1 solution into a 384-well white OptiPlate™-384 (2 nM final concentration).
- Add 2.5 µL of 4X U*Light*™-Crosstide (50 nM final concentration).
- Add 2.5 µL of 4X ATP mix (1 mM to 10 nM final concentrations).
- Cover plate with TopSeal-A and incubate 60 min at 23°C.
- Add 5 µL of 4X Stop Solution and incubate 5 min at 23°C.
- Add 5 µL of Detection Mix (2 nM Eu-anti-phospho-Crosstide antibody final concentration) and mix.
- Cover plate with TopSeal-A and incubate 60 min at 23°C.
- Remove TopSeal-A and read in TR-FRET mode at 665 nm (excitation at 320 or 340 nm).

Typical Product Data

MSK1 kinase assay using ULight™-Crosstide and Eu-anti-phospho-Crosstide antibody obtained using the EnVision® Multilabel Reader:



Suggested Materials

	Supplier
• Substrate: U*Light*™- Crosstide	Revvity Inc
• Antibody: Eu- anti-phospho-Crosstide (GSK-3α Ser21)	Revvity Inc
• Kinase: MSK1, active	Millipore/Upstate
• Detection Buffer: LANCE® Detection Buffer, 10X	Revvity Inc
• Plate: OptiPlate™-384, white	Revvity Inc
• TopSeal™-A	Revvity Inc

TRF0202-R Rev01

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